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Pharmacologic Comparison of Selected Agonists for the M_1 Muscarinic Receptor in Transfected Murine Fibroblast Cells (B82)[1]

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ABSTRACT

The radioligand binding and functional properties of 10 muscarinic agonists for the M_1 muscarinic receptors were characterized on the murine fibroblast B82 cells, which have been transfected with the m_1 gene. All of the muscarinic agonists completely inhibited [^{3}H](−)methyl-3-quinuclidinyl benzilate binding to the M_1 muscarinic receptor in the transfected B82 cells. Their apparent inhibition constant values for agonist/[^{3}H](−)methyl-3-quinuclidinyl benzilate inhibition experiments correlate well with their EC_{50} values in stimulating phosphatidylinositide hydrolysis. Based on the maximal functional effects: (+)-cismethyl-dioxolane, oxotremorine-M, acetylcholine, carbachol and methacholine are most efficacious, McN-A-343 and arecoline are least efficacious, whereas the efficacies of oxotremorine and pilocarpine are intermediate. In addition, McN-A-343 inhibited carbachol-stimulated phosphatidylinositide hydrolysis. Spare receptors were detected for oxotremorine-M, methacholine and carbachol, but not the rest of the agonists, by comparing the receptor-occupancy curves with the concentration-response curves. These results suggest that the presence of a quaternary nitrogen (trimethylammonium group) within the structure of the agonist may be important for the expression of full agonist activity.

Recent studies have demonstrated that there are at least three pharmacologic types of muscarinic receptors (M_1 to M_3) (for review see Mei *et al.*, 1989c). The genes for some of the muscarinic receptor types (m_1 to m_5) have been cloned (Kubo *et al.*, 1986a, 1986b; Peralta *et al.*, 1987; Bonner *et al.*, 1987). Studies of the expression of these genes by either *in situ* hybridization or Northern blots have demonstrated that the muscarinic receptor types are distributed throughout the central and peripheral nervous systems. Birdsall *et al.* (1978) studied the interaction of muscarinic agonists with the muscarinic receptors in rat cerebral cortex and found three classes of binding sites with different affinities for agonists (superhigh, high and low) although these binding sites had similar affinities for the antagonists. The low-affinity agonist site in the cerebral cortex and hippocampus was shown to have high affinity for pirenzepine, an M_1 selective antagonist, and the superhigh and high-affinity site had lower affinity for pirenzepine (Birdsall and Hulme, 1983). However, a mixture of more than one type of muscarinic receptor in a defined tissue has hampered a detailed pharmacologic characterization of the interaction of muscarinic agonists with a muscarinic receptor type.

Recently, we obtained a eukaryotic expression system that expresses only the M_1 muscarinic receptors, *i.e.*, murine fibroblast B82 cells which have been transfected with the m_1 gene (Lai *et al.*, 1988). The signal transduction systems for this M_1 muscarinic receptor type have been characterized (Mei *et al.*, 1989a, 1989b; Lai *et al.*, 1990). The M_1 muscarinic receptor is coupled to the hydrolysis of inositol lipids through guanyl nucleotide binding proteins. Subsequently, Ca^{++} mobilization after activation of the receptor was detected (Lai *et al.*, 1990). We have demonstrated that spare receptors exist for carbachol in stimulating PI hydrolysis in the cells expressing higher receptor concentrations (Mei *et al.*, 1989b). In this study, we characterized the interaction of 10 muscarinic agonists with the M_1 muscarinic receptor in the transfected B82 cells. The agonist/[^{3}H](−)MQNB competition experiments showed that most muscarinic agonists with a quaternary nitrogen (tri-

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ABBREVIATIONS: PI, phosphatidylinositide; IMDM, Iscove's modified Dulbecco's medium; [^{3}H](−)MQNB, [^{3}H](−)methyl-3-quinuclidinyl benzilate; [^{3}H]IP_1, [^{3}H]inositol monophosphates; K_H, dissociation constant of high-affinity state; CD, (+)-cismethyl-dioxolane; OXO-M, oxotremorine-M; McN-A-343, (4-hydroxy-2-butynyl)-1-trimethylammonium-*m*-chlorocarbanilate chloride; E_{max}, maximal accumulation of [^{3}H]inositol monophosphates; K_i, inhibition constant; η_H, Hill coefficient; K_L, dissociation constant of low-affinity state; $K_{0.5}$, inhibition constant when the Hill coefficient is less than 0.5.

methylammonium group) bound to the M_1 receptor with two affinities, whereas three agonists with a tertiary nitrogen bound to the M_1 receptor with a single affinity state. Functional correlations of these affinities were studied. For the full agonists, the K_H value correlated well with the EC_{50} values. In general, the functional EC_{50} value correlated well with the K_i values for all agonists studied.

Materials and Methods

Cell culture. The m_1 muscarinic receptor gene under the constitutive control of the human β-actin promoter in the vector pHβAPr-1-neo (Gunning *et al.*, 1987) was transfected into the murine fibroblast (B82) cells (Lai *et al.*, 1988, 1990). A clone, designated as LK3–3, which expressed the highest density of muscarinic receptors from a single transfection experiment, was maintained in Costar 75-cm^2 tissue culture flasks in a medium containing 45% Dulbecco's modified Eagle's medium, 45% Ham's F-12 medium, 5% fetal bovine serum, 5% newborn calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin, and incubated in a humidified atmosphere of 95% air and 5% CO_2, at 37°C. The cells were seeded into 2-cm^2 wells at a concentration of 125,000 cells/well 24 h before experiments.

Radioligand binding assays. [^{3}H](−)MQNB (70 Ci/mmol; New England Nuclear, Boston, MA) binding to the intact cells was performed as described previously (Mei *et al.*, 1989a). For drug/[^{3}H](−)MQNB competition assays, the cells were incubated with 10 concentrations of indicated agonists or antagonists and 300 to 400 pM [^{3}H](−)MQNB in 1 ml of IMDM for 3 h under the same conditions. Physostigmine (10 μM) was included in the assay medium containing acetylcholine. The incubation was terminated by aspirating the medium and placing the cell culture trays on ice. The cells were rinsed once with 1 ml of ice-cold IMDM for 10 min at 4°C, and then solubilized with 0.5 ml of 1% Triton X-100, scraped and transferred to scintillation vials. Scintillation fluid was added and the samples were counted by a liquid scintillation spectrophotometer (Searle Analytic 81) with 44% efficiency.

Inositol lipid hydrolysis studies. The experiments were done as described previously (Mei *et al.*, 1989a). Cells were routinely incubated with 0.2 μM myo-[2,^{3}H]inositol in 0.5 ml of IMDM with 95% air-5% CO_2 at 37°C for 20 to 22 h. At the end of the incubation, the medium was replaced with 1 ml of fresh IMDM 10 min before starting 10 mM lithium chloride treatment for 10 min. Various concentrations of agonists were then added to initiate the reaction. The reaction was terminated 60 min later by aspirating the medium and adding 0.31 ml of methanol. The cells in methanol were scraped off from the wells with a Costar cell scraper (Costar, Cambridge, MA) and collected into chloroform-resistant tubes (American Scientific Products, McGaw Park, IL). Another 0.31 ml of methanol was used to wash each of the wells and the remaining cells were collected. Each cell suspension was mixed with 0.62 ml of chloroform and 0.31 ml of double-distilled water and mixed vigorously for 15 sec. After the phases were separated by centrifugation at 1000 × *g* for 5 min, a 0.7-ml aliquot of the upper aqueous phase was added to 2 ml of distilled water. The mixture was passed through mini-columns (Bio-Rad Laboratories, Richmond, CA) consisting of 2 ml of 10% slurry of anion exchange resin in formate form (AG 1-X8, 100–200 mesh; Bio-Rad Laboratories, Richmond, CA). Each column was then washed five times with 5 ml of distilled water. The [^{3}H]IP_1 was eluted by 2 ml of 0.2 M ammonium formate/0.1 M formic acid and collected into a scintillation vial. Nine milliliters of AquaMix (ICN Radiochemicals, San Diego, CA) were added and the radioactivity was counted.

Data analyses. All the binding and concentration-response data were analyzed by a computerized nonlinear least-squares regression program developed by Susan Yamamura for the Apple II Computer (Regan *et al.*, 1981). The *F* values of comparing the one-site *vs.* two-site fit were calculated as described previously (Mei *et al.*, 1989b). The K_i value ($K_{0.5}$ when $n_H < 1$), K_H value, or K_L value was corrected from the IC_{50} value using the Cheng and Prusoff (1973) equation. Statistical differences were analyzed using the one-way analysis of variance for grouped data (Tallarida and Murray, 1987) followed by the paired *t* test. A value of $P < .05$ was considered statistically significant.

Materials. CD was from Research Biochemicals, Inc. (Wayland, MA); OXO-M, McN-A-343 and methoctramine were from Research Biochemicals, Inc. (Natick, MA). Himbacine was a gift from Dr. W. L. Taylor; 4-DAMP was a gift from Dr. R. Barlow; pirenzepine and AF-DX 116 were gifts from Dr. Karl Thomae, Gmbh; hexahydro-siladifenidol was a gift from Dr. G. Lambrecht. Physostigmine and the other muscarinic ligands are from Sigma (St. Louis, MO). The structures of the 10 agonists are shown in figure 1.

Results

Inhibition of specific [^{3}H](−)MQNB binding by muscarinic agonists to the M_1 muscarinic receptor in the transfected B82 cells. All of the 10 selected muscarinic agonists inhibited specific [^{3}H](−)MQNB binding to the M_1 muscarinic receptor in intact transfected B82 cells by 100%, however, with different affinities. The IC_{50} values of the agonists ranged from 4.4 μM for oxotremorine to 760 μM for bethanechol. The shapes of the agonist/[^{3}H](−)MQNB competition curves were steep for pilocarpine, arecoline and oxotremorine with Hill coefficients close to unity. The agonist/[^{3}H](−)MQNB competition curves for the rest of the agonists are shallow with Hill coefficients significantly less than one, and are better fitted by a nonlinear least-squares regression analysis with a two-site model ($P < .05$, *F* test). The K_i values, K_H values, or values calculated from the corresponding IC_{50} values are shown in table 1.

Stimulation of the hydrolysis of inositol lipids in the transfected B82 cells by muscarinic agonists. All of the muscarinic agonists stimulated hydrolysis of inositol lipids as evidenced by increased accumulation of [^{3}H]IP_1 by the agonists in the presence of 10 mM lithium chloride. The maximal stimulation of the agonists is different. The E_{max} varied from 120,000 cpm/10^6 cells for CD, OXO-M, acetylcholine and carbachol to 15,000 cpm/10^6 cells for McN-A-343. Accordingly, CD, OXO-M, acetylcholine, carbachol and methacholine are the most efficacious agonists in stimulating M_1 receptors for the hydrolysis of inositol lipids in the transfected B82 cells. Arecoline and McN-A-343 are the least efficacious. The most potent drugs with EC_{50} values of 1.1 to 4.4 μM include oxotremorine, OXO-M, CD, acetylcholine and arecholine; the least potent drug was bethanechol with an EC_{50} value of 240 μM, while methacholine, McN-A-343 and pilocarpine are intermediate with EC_{50} values of 8.2 to 11 μM (table 2).

The agonist/[^{3}H](−)MQNB inhibition curves were corrected by the Cheng and Prusoff (1973) equation and compared with the concentration-response curves. Although the 10 agonists studied all have larger IC_{50} values (legend to table 1) than EC_{50} values (table 2), only three agonists (OXO-M, carbachol and methacholine) displayed EC_{50} values significantly smaller than the $K_{0.5}$ values. As indicated in tables 1 and 2, there was no overlap between the ranges of the $K_{0.5}$ values and the corresponding EC_{50} values. Thus, a significant number of spare receptors are readily demonstrated in this clone for these three agonists.

A significant linear correlation was observed between the $K_{0.5}$ or K_i values of the agonists and the EC_{50} values ($r = 0.99$, $P < .01$). There is also a significant positive correlation between the percentages of the high-affinity state for agonists and the

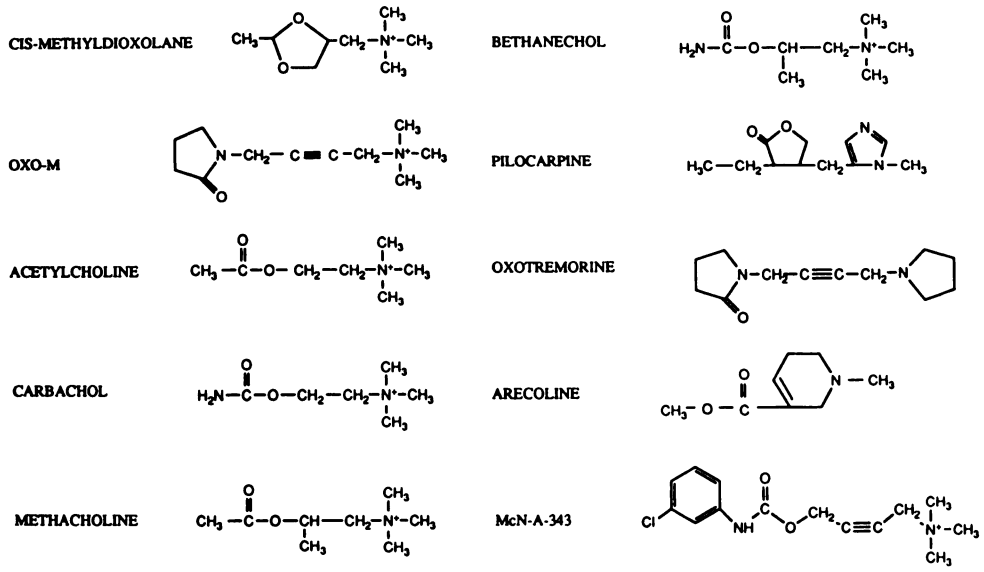

Fig. 1. Structures of the 10 muscarinic agonists used in this study.

TABLE 1

Inhibition of [^{3}H](−)MQNB binding to the M_1 muscarinic receptors in the transfected B82 cells by muscarinic agonists[a]

Agonist	One Site[b]		Two Sites[c]		
	K_I[d]	Hill Coefficient	K_H	High Affinity State	K_L[e]
	μM		μM	%	μM
CD	1.9 (0.72–4.2)	0.78 ± 0.02	0.57 (0.31–1.0)	53 ± 8.1	7.1 (4.7–10)
OXO-M	2.5 (2.0–3.2)	0.79 ± 0.03	0.58 (0.46–1.2)	44 ± 2.6	6.4 (5.2–7.1)
Acetylcholine	3.4 (3.0–3.9)	0.71 ± 0.04	0.92 (0.70–1.3)	55 ± 1.8	14 (12–18)
Carbachol	29 (28–31)	0.83 ± 0.02	17 (11–27)	70 ± 14	110 (87–130)
Methacholine	23 (18–34)	0.85 ± 0.02	14 (11–16)	75 ± 5.6	97 (77–130)
Bethanechol	190 (160–240)	0.79 ± 0.05	28 (8.4–93)	29 ± 8.2	280 (270–300)
Pilocarpine	12 (9.7–14)	0.92 ± 0.07	—	—	—
Oxotremorine	1.1 (0.71–2.8)	1.0 ± 0.02	—	—	—
Arecoline	4.1 (3.5–5.6)	1.3 ± 0.04	—	—	—
McN-A-343	8.2 (7.8–8.6)	0.80 ± 0.05	0.09 (0.01–0.70)	14 ± 6.8	11 (8.4–16)

[a] The agonist/[^{3}H](−)MQNB competition experiments were performed as described in "Materials and Methods." The concentrations of the agonists used were: 1 nM to 0.1 mM for CD, and 0.1 nM to 0.1 mM for the other drugs.

[b] The data were analyzed by a computer-generated nonlinear least-squares regression program for a four parameter logistic equation. The IC_{50} values are 7.4 (2.8–16) μM for CD, 10 (7.6–13) μM for OXO-M, 13 (12–15) μM for acetylcholine, 110 (110–120) μM for carbachol, 91 (71–130) μM for methacholine, 760 (630–950) μM for bethanechol, 47 (38–55) μM for pilocarpine, 4.4 (2.8–11) μM for oxotremorine, 16 (13–22) μM for arecoline and 32 (30–33) μM for McN-A-343.

[c] The data were analyzed by a computer-generated nonlinear least-squares regression program for two-site model with three parameters. The IC_{50} values for the high and low affinity states are 2.2 (1.2–4.0) μM and 28 (18–41) μM for CD, 2.3 (1.4–4.8) μM and 25 (20–28) μM for OXO-M, 3.6 (2.7–5.0) μM and 55 (47–72) μM for acetylcholine, 56 (43–64) μM and 380 (301–508) μM for methacholine, 67 (42–110) μM and 420 (340–510) μM for carbachol, 110 (33–360) μM and 1100 (1000–1200) μM for bethanechol and 0.35 (0.03–2.7) μM and 42 (33–61) μM for McN-A-343.

[d] $K_{0.5}$ values for CD, OXO-M, acetylcholine, carbachol, bethanechol and McN-A-343 since the n_H values for the agonist/[^{3}H](−)MQNB competition curves are significantly less than one.

[e] K_L, dissociation constant of low-affinity state.

E_{max} values (r = 0.74, $P < .05$), suggesting that the agonist-stimulated [^{3}H]IP_1 accumulation may be dependent on the high affinity state of the M_1 muscarinic receptors in the transfected B82 cells. In contrast, a significant negative correlation was observed between the percentages of the low-affinity state for agonists and the E_{max} values (r = −0.71, $P < .05$). The correlation between the IC_{50}/EC_{50} ratio and the E_{max} values did not reach significance.

Inhibition of carbachol-stimulated [^{3}H]IP_1 accumulation in the transfected B82 cells by McN-A-343. Since McN-A-343 is the least efficacious drug among the studied agonists, we investigated the possible inhibitory effects of McN-A-343 on [^{3}H]IP_1 accumulation stimulated by 100 μM carbachol in the transfected B82 cells. McN-A-343 inhibited [^{3}H]IP_1 accumulation with an IC_{50} value of 66 μM (37–94 μM) (fig. 2).

Inhibition of specific [^{3}H](−)MQNB binding to M_1 muscarinic receptors by antagonists in the transfected B82 cells. Seven muscarinic antagonists inhibited specific [^{3}H](−)MQNB binding to M_1 muscarinic receptors in the transfected B82 cells. The order of their potencies is compatible with

TABLE 2

Stimulation of [^{3}H]IP$_1$ accumulation by muscarinic agonists in the transfected B82 cells[a]

	EC_{50}[b]	E_{max}[c]	Hill Coefficient
	μM	cpm/10^6 cells	
CD	2.0 (1.8–2.4)	120,000 ± 8,800	1.0 ± 0.06
OXO-M	1.1 (0.74–1.4)	120,000 ± 4,000	1.1 ± 0.19
Acetylcholine	2.9 (1.6–4.6)	120,000 ± 6,000	1.2 ± 0.11
Carbachol	13 (11–16)	110,000 ± 6,800	1.1 ± 0.05
Methacholine	8.2 (5.6–15)	100,000 ± 10,000	1.1 ± 0.05
Bethanechol	240 (160–370)	88,000 ± 2,200	0.84 ± 0.10
Pilocarpine	11 (6.6–19)	33,000 ± 400	1.1 ± 0.22
Oxotremorine	1.1 (0.64–2.0)	32,000 ± 1,600	0.97 ± 0.08
Arecoline	4.4 (1.2–11)	17,000 ± 4,200	1.1 ± 0.10
McN-A-343	9.0 (4.4–14)	15,000 ± 2,400	0.97 ± 0.03

[a] The [^{3}H]IP$_1$ accumulation studies were performed as described in "Materials and Methods." The data were analyzed by a computer-generated nonlinear least squares regression program for one-site model with three parameters.

[b] The EC_{50} values are shown as geometric means with the range in parentheses. The rank order of EC_{50} values is oxotremorine = OXO-M = CD = carbachol = arecoline < methacholine = McN-A-343 = pilocarpine = carbachol < bethanechol ($P < .05$, one-way analysis of variance).

[c] The E_{max} values are shown as the stimulated minus basal [^{3}H]IP$_1$ accumulation. The basal [^{3}H]IP$_1$ accumulation was 24,000 ± 3900 cpm/10^6 cells. The rank order of E_{max} values is CD = OXO-M = acetylcholine = carbachol = methacholine ≥ bethanechol > pilocarpine = oxotremorine > arecoline = McN-A-343 ($P < .05$, one-way analysis of variance).

the previous assignment of M_1 to the muscarinic receptors (Mei *et al.*, 1989a).

Discussion

This study characterized the binding and the functional properties of 10 muscarinic agonists on the M_1 muscarinic receptor in the transfected B82 cells with the following major findings. 1) The agonists demonstrate a spectrum of maximal effects (E_{max} values) in stimulating PI hydrolysis. CD, OXO-M, acetylcholine, carbachol and methacholine are full agonists, whereas McN-A-343 and arecoline are least efficacious, with oxotremorine, pilocarpine and bethanechol in between. 2) The maximal [^{3}H]IP$_1$ accumulation initiated by McN-A-343, a commonly reported "M_1 selective" agonist, was only 5% of that by carbachol. Furthermore, McN-A-343 inhibited carbachol-stimulated hydrolysis of inositol lipids in these cells. 3) Seven muscarinic agonists that contained a quaternary nitrogen, CD, OXO-M, acetylcholine, carbachol, methacholine, bethanechol and McN-A-343, bound to the M_1 muscarinic receptor in intact transfected cells with high and low affinities, whereas the rest of the agonists bound with a single affinity. Furthermore, spare receptors were detected for OXO-M, methacholine as well as for carbachol but not for the rest of the agonists.

The LK3-3 cells are one of the seven clones of cells transfected with the m_1 muscarinic receptor gene and express the highest [^{3}H](−)MQNB binding capacity with a B_{max} value of 240 fmol/10^6 cells (Mei *et al.*, 1989a). Each of the 10 agonists fully inhibited [^{3}H](−)MQNB binding to the intact transfected cells. The displacement of [^{3}H](−)MQNB binding by these agonists indicates that they bind to M_1 muscarinic receptors. The affinities of these agonists for the M_1 muscarinic receptor were different (table 2). The linear correlation of the EC_{50} values of the agonists in stimulating PI hydrolysis with their K_i values, or $K_{0.5}$ values, suggest that these agonists mediate the response by interacting with the M_1 muscarinic receptors in the transfected B82 cells. The functional state of the M_1 muscarinic receptors in these cells may be the one with higher affinity for the agonists since the percentages of the high-affinity state for agonists demonstrated a significant positive correlation with the E_{max} values. However, this relationship was seen more clearly for carbachol in murine fibroblast cells expressing different receptor densities (Mei *et al.*, 1989b). A full occupancy of the M_1 muscarinic receptors in these cells by some agonists may not produce a maximal response. Functional studies of the hydrolysis of inositol lipids demonstrated various efficacies of these agonists. The most efficacious agonists are CD, OXO-M, acetylcholine, carbachol and methacholine, whereas McN-A-343 and arecoline are the least efficacious. Oxotremorine, pilocarpine and bethanechol are intermediate in

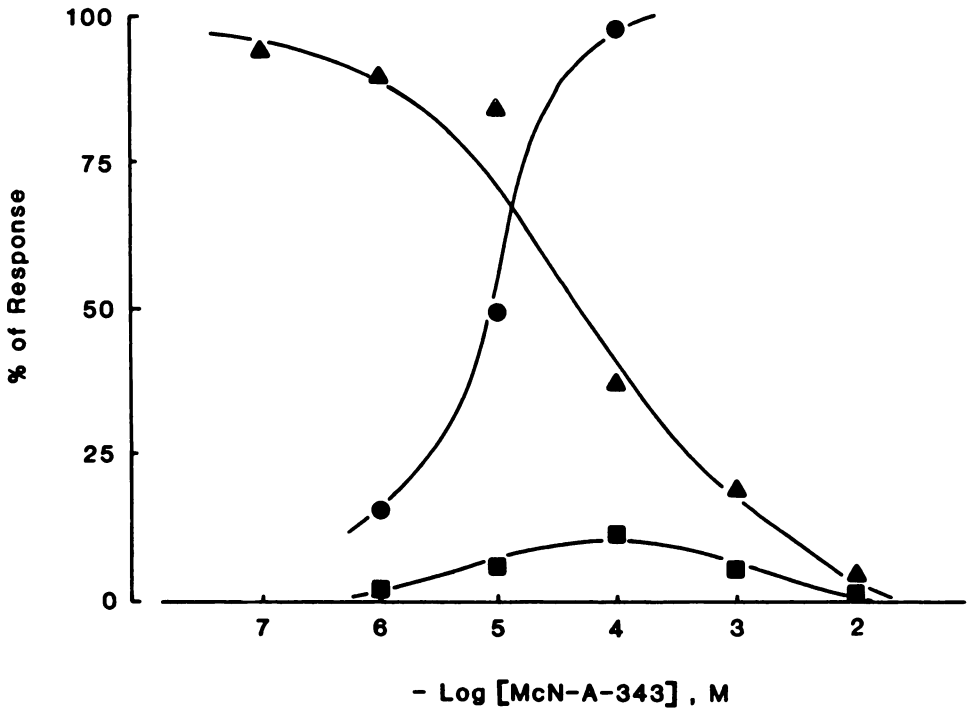

Fig. 2. Concentration-response curves of McN-A-343 in stimulating [^{3}H]IP$_1$ accumulation (●, ■) and in inhibiting carbachol-stimulated [^{3}H]IP$_1$ accumulation (▲) in the transfected B82 cells. ●, Concentration-response curve normalized by the E_{max} value of McN-A-343 (as 100%); ■, concentration-response curve normalized by 100 μM carbachol-stimulated [^{3}H]IP$_1$ accumulation as (100%). Data shown represent means of three or more separate experiments with the S.E.M. value less than 10%. The inhibition experiments were performed by adding various concentrations of McN-A-343 in IMDM, where 100 μM carbachol was added to stimulate [^{3}H]IP$_1$ accumulation.

TABLE 3
Inhibition of [^{3}H](−)MQNB binding to the M_1 muscarinic receptors in the transfected B82 cells by muscarinic antagonists*

	IC_{50}	K_i	n_H
	nM	nM	
Atropine	1.4 (1.3–1.6)	0.51 (0.28–0.74)	1.0 ± 0.04
4-DAMP	3.7 (3.1–4.9)	1.2 (0.44–2.2)	0.94 ± 0.04
Pirenzepine	37 (25–63)	13 (5.1–28)	1.0 ± 0.01
Hexahydro-sila-difenidol	46 (26–71)	14 (9.2–20)	0.98 ± 0.04
Himbacine	160 (100–220)	52 (24–99)	0.99 ± 0.10
Methoctramine	1100 (440–1600)	310 (210–580)	1.2 ± 0.04
AF-DX 116	1500 (910–3500)	540 (410–720)	0.97 ± 0.09

* The antagonist [^{3}H](−)MQNB competition experiments were performed as described in "Materials and Methods." The data were analyzed by a computer-generated nonlinear least-squares regression program for a logistic model with four parameters. The IC_{50} values were converted to K_i values by using the Cheng and Prusoff (1973) equation.

stimulating the hydrolysis of inositol lipids in the transfected B82 cells. In the cell lines N1E-115 and SK-N-SH, in which M_2 or M_3 muscarinic receptors predominate, OXO-M was found to be a full agonist in stimulating inositol phosphate formation while bethanechol, arecoline, oxotremorine, pilocarpine and McN-A-343 were partial agonists (Fisher and Snider, 1987). Comparing our data with those of Fisher and Snider (1987), a significant linear correlation was obtained between the maximal responses of these five partial agonists in the LK3-3 cells and in SK-N-SH cells ($r = 0.95$, $P < .01$), or in N1E-115 cells ($r = 0.88$, $P < .05$), suggesting that the efficacies of the muscarinic agonists in stimulating PI hydrolysis may not depend on the structures of the muscarinic receptor subtypes. Consequently, none of these agonists is likely to be very selective for M_1, M_2 or M_3 receptors.

We have shown that spare M_1 muscarinic receptors exist for the agonist carbachol in the LK3-3 cells. Carbachol needs to occupy only 14% of the total receptors to initiate a half-maximal effect (Mei *et al.*, 1989b). When the receptor occupancy curves by these agonists were corrected by using the Cheng and Prusoff equation (1973) and compared with the concentration-response curves, the concentration-response curves of agonists OXO-M, methacholine and carbachol are observed on the left side of the occupancy curves. The $K_{0.5}$ values are different from the EC_{50} values, confirming that there are spare receptors for these agonists in the LK3-3 cells. It is interesting that spare receptors were not detected for the other agonists by comparing the corrected receptor-occupancy curves with the concentration-response curves. The limited availability of guanyl nucleotide binding proteins and/or second messenger systems in the transfected B82 cells may also be an important determinant of spare receptors (Mei *et al.*, 1989b). Furthermore, the expression or detection of spare receptors may be dependent on the intrinsic structural properties of the agonists.

Binding selectivity of the muscarinic antagonist pirenzepine could be influenced by the lipids in the environment of the receptor molecules (Mei *et al.*, 1987; Berstein *et al.*, 1989). The results of this study do not address the role of the microenvironment of the receptor molecules in the expression of agonist binding. In addition, the intrinsic structural properties of the muscarinic agonists may be important for the expression of activity of individual agonists. The importance of the quaternary nitrogen for the expression of full agonist's activity confirms the model reviewed by Ehlert *et al.*, 1983. The antagonist binding results (table 3) also confirm this model since maximum cholinolytic potency is usually associated with the presence of alkyl or aromatic rings on the acyl moiety of anticholinergic esters. Thus, the activity of McN-A-343 can be rationalized by the presence of the bulky substitution of the acyl group, which markedly reduced the efficacy associated with the trimethylammonium group.

McN-A-343 was originally reported to increase blood pressure via the muscarinic receptor in the sympathetic ganglia and had little or no effect on other peripheral organs (Roszkowski, 1961). It was later reported to be a selective agonist for the M_1 muscarinic receptor when evidence for heterogeneity of muscarinic receptors became available (Goyal and Rattan, 1978; Hammer and Giachetti, 1982). In this study, McN-A-343 is an agonist with low efficacy for the M_1 muscarinic receptors, although it bound to the receptors with comparably high affinity. Compatible with the behavior of a partial agonist, McN-A-343 inhibited carbachol-stimulated [^{3}H]IP_1 accumulation (fig. 2). Both the muscarinic receptors in the brain and those in the sympathetic ganglia have high affinity for pirenzepine and have been classified as the M_1 muscarinic receptors (Hammer *et al.*, 1980; Hammer and Giachetti, 1982; for review, see Mei *et al.*, 1989c). The corrected occupancy curve of McN-A-343, superimposed with its concentration-response curve (fig. 2), suggests a positive correlation between the receptor occupancy and its stimulatory response.

The M_1 muscarinic receptor in the transfected B82 cells has high affinity for pirenzepine and low affinity for AF-DX 116 (Mei *et al.*, 1989a). We have further studied the M_1 muscarinic receptor in the LK3-3 cells by characterizing the binding activity of selected muscarinic antagonists toward the M_1 receptor. In addition to pirenzepine, hexahydro-sila-difenidol and 4-diphenylacetoxy-*N*-methylpiperidine-methiodide had high affinity for these M_1 receptors. The M_2 selective antagonists methoctramine and himbacine showed a low affinity. The affinities of some muscarinic antagonists for the M_1 muscarinic receptors in the LK3-3 cells are in agreement with those obtained by Buckley *et al.* (1989) except for methoctramine. The K_i value of methoctramine in the LK3-3 cells was 310 nM, about 20-fold higher than the K_i value (of 16 nM) in CHO-Kl cells (Buckley *et al.*, 1989). Methoctramine was found to be a selective antagonist for the M_2 muscarinic receptors (Melchiorre *et al.*, 1987a, 1987b). The affinity of methoctramine observed in this study is in better agreement with such a selectivity.

In conclusion, we have used LK3-3 cells, a cell line transfected with only the M_1 muscarinic receptor as a model system to study the efficacy and binding properties of 10 muscarinic agonists. The results suggest that the presence of a quaternary nitrogen (trimethylammonium group) within the structure of

the agonists may be important for the expression of full agonist activity.

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